Full Paper

Effect of Mexiletine on Fenvalerate-Induced Nociceptive Response in Diabetic Mice

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Abstract. The effect of mexiletine on the nociceptive behavior induced by the intrathecal injection of fenvalerate, which predominantly activates tetrodotoxin-resistant (TTX-R) sodium channels, was studied in diabetic mice. The intrathecal injection of fenvalerate induced a characteristic behavioral syndrome that mainly consisted of reciprocal hind limb scratching directed toward caudal parts of the body and biting or licking of the hind legs in mice. The intensity of fenvalerate-induced nociceptive responses was significantly greater in diabetic mice than non-diabetic mice. This fenvalerate-induced behavior was dose-dependently inhibited by mexiletine (3–30 mg/kg, i.p.). Intrathecal pretreatment with fenvalerate produced thermal hyperalgesia and allodynia in the tail-flick test in naive mice. Furthermore, mexiletine at doses of 10 and 30 mg/kg, i.p., dose-dependently and significantly reduced fenvalerate-induced thermal hyperalgesia and allodynia in the tail-flick test in naive mice. These present data suggest that i.p. pretreatment with mexiletine produced dose-dependent inhibition of fenvalerate-induced hyperalgesia and allodynia in mice, especially diabetic mice. This effect may be, at least in part, mediated by the inhibition of TTX-R sodium channel-mediated nociceptive transmission in the spinal cord.

Keywords: mexiletine, allodynia, hyperalgesia, tetrodotoxin-resistant sodium channel, diabetes

Opening of voltage-gated sodium channels causes a rapid depolarization of the membrane potential and constitutes the upstroke phase of the action potential. Most neuronal sodium channels can be blocked by tetrodotoxin (TTX) with K_d values of 1–10 nM. However, sodium channels that are resistant to the blocking action of TTX have been found in both the central and peripheral nervous systems (1–3). Dorsal root ganglion neurons express a slow activating and inactivating TTX-resistant (TTX-R) sodium channel as well as a fast activating and inactivating TTX-sensitive (TTX-S) sodium channel (4–7). The TTX-R sodium channel is predominantly expressed in the capsaicin-sensitive small neurons of the dorsal root ganglion and appears to play an important role in nociceptive transmission (8–13), particularly in allodynia and hyperalgesia (14). Furthermore, several lines of evidence indicated that the function of TTX-R sodium channels are

increased in early diabetic neuropathy and, therefore, may contribute to the pathophysiology of painful diabetic neuropathy (15, 16). In addition, algogenic mediators such as prostaglandin, adenosine, and serotonin enhance TTX-R sodium current (17–19). Null mutant mice for TTX-R sodium channels show antinociception in response to noxious mechanical stimuli and a delayed development of inflammatory hyperalgesia (20). In spinal cord astrocytes, protein kinase C enhanced TTX-R sodium channels with leftward shifts in the voltage-dependence of both activation and inactivation and produced faster kinetics (21). Several studies using protein kinase C γ -knockout mice (22) and diabetic animals (23–25) have indicated that protein kinase C may play important roles in hyperalgesia.

The pyrethroid insecticides are known to modulate the gating kinetics of neuronal sodium channels to cause repetitive discharges and membrane depolarization. In TTX-R sodium channels, fenvalerate, a type II pyrethroid, irreversibly prolongs the sodium current

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during depolarization and greatly augments and prolongs the tail current (26). We previously demonstrated that the intrathecal (i.t.) injection of fenvalerate induced a characteristic behavioral syndrome that mainly consisted of reciprocal hind limb scratching directed toward caudal parts of the body and biting or licking of the hind legs in both non-diabetic and diabetic mice (27). However, the intensity of such fenvalerate-induced nociceptive responses was significantly greater in diabetic mice than in non-diabetic mice (27). Calphostin C (3 pmol, i.t.), a selective protein kinase C inhibitor, significantly inhibited intrathecal fenvalerate-induced nociceptive behavior, with a rightward shift of the dose-response curve to the level observed in non-diabetic mice (27). On the other hand, when non-diabetic mice were pretreated with phorbol-12,13-dibutyrate (50 pmol, i.t.), the dose-response curve for intrathecal fenvalerate-induced nociceptive behavior was shifted leftward to the level observed in diabetic mice (27). Based on these results, we suggested that the sensitization of sodium channels, probably TTX-R sodium channels, by the long-term activation of protein kinase C may play an important role in the enhancement of the duration of fenvalerate-induced nociceptive behavior in diabetic mice (27). Furthermore, we also showed that MK-801, an NMDA-receptor antagonist, when given i.t. before fenvalerate caused a dose-dependent inhibition of fenvalerate-induced nociceptive responses (28). Moreover, the fenvalerate-induced nociceptive response was inhibited by GR82334, a non-peptidic NK-1-receptor antagonist (28). These results led us to suggest that the fenvalerate-induced nociceptive response may be mediated through the release of glutamate and neurokinins, which causes the activation of NMDA and NK-1 receptors, by affecting sodium channels (28).

Dejgard et al. (29) reported that mexiletine, a lidocaine-like antiarrhythmic drug, significantly relieves symptoms in painful diabetic neuropathy. We previously suggested that the antinociceptive effect of mexiletine in diabetic mice may involve the inhibition of substance P-mediated nociceptive transmission in the spinal cord (30, 31). Although it is reasonable to speculate that mexiletine-induced antinociception and blockade of sodium channels may somehow be related, the detailed mechanisms that underlie the antinociceptive effect of mexiletine in painful diabetic neuropathy remain unknown.

Therefore, the aim of this study was to investigate the effects of mexiletine on fenvalerate-induced nociception in diabetic mice to determine whether such effects involve sodium channel blockade.

Materials and Methods

Animals

Male ICR mice (Tokyo Laboratory Animals Science Co., Tokyo), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at $24 \pm 1^\circ\text{C}$ with a 12-h light-dark cycle. The animals were rendered diabetic by an injection of streptozotocin (200 mg/kg, i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with the vehicle alone. The experiments were conducted 2 weeks after the injection of streptozotocin or vehicle. Mice with serum glucose levels above 4000 mg/l were considered diabetic. This study was carried out in accordance with the Declaration of Helsinki and with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology.

Nociceptive responses

The experiment was performed according to the method described by Hylden and Wilcox (32). Each mouse was acclimated to an acrylic observation chamber ($39 \times 26 \times 24 \text{ cm}^3$) for at least 5 min before the injection of an fenvalerate. Immediately after the injection, the mice were placed in the observation chamber. The cumulative duration (s) of biting, paw licking, and scratching episodes was measured for 30 min after the injection of fenvalerate.

Thermal allodynia and hyperalgesia

The nociceptive response was evaluated by recording the latency to withdrawal of the tail in response to several different rates of noxious skin heating, as previously described (24). Briefly, the tails of mice were exposed to a focused beam of light from a 50-W projection bulb. The heat intensity was set to one of five values by adjusting the source of voltage for the bulb from 35 and 50 V. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured electronically. In the absence of a response up to a predetermined maximum latency (30 s), the trial was terminated to prevent tissue damage. The behavioral response induced by fenvalerate at a dose of $1 \mu\text{g}$ peaked at 10 to 20 min and completely disappeared within 60 min after injection. Therefore, in subsequent experiments the tail-flick latency was determined before and 60 min after the injection of fenvalerate. The heat intensities at 35 and 50 V produced surface skin heating rates of 0.4 and 0.9°C/s , respectively.

I.t. injection

I.t. administration was performed following the method described by Hylden and Wilcox (33). Each i.t. injection was administered using a 30-gauge needle directly through the intact skin between the L5 and L6 vertebrae. Drugs were given in a volume of 5 μ l/mouse.

Drugs

Fenvalerate was purchased from Calbiochem-Novabiochem Corporation (La Jolla, CA, USA). Mexiletine hydrochloride was generously supplied by Boehringer Ingelheim KG (Ingelheim, Germany). Fenvalerate was dissolved in 0.4% DMSO in saline. Mexiletine was dissolved in saline and administered i.p. or i.t. 30 min prior to the injection of fenvalerate.

Data analysis

The data are expressed as means $\pm$ S.E.M. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Bonferroni/Dunn test.

Results

Behavioral response induced by i.t. administered fenvalerate

Intrathecal injection of fenvalerate elicited a characteristic behavioral syndrome that mainly consisted of reciprocal hind limb scratching directed towards caudal parts of the body and biting or licking of the hind legs in mice. In diabetic mice, i.t. injection of fenvalerate elicited a characteristic nociceptive behavioral syndrome, as in non-diabetic mice. However, the intensity of the fenvalerate-induced nociceptive response was significantly greater in diabetic mice than in non-diabetic mice. In diabetic mice, the duration of the nociceptive behavioral response induced by fenvalerate at a dose of 0.1 μ g, i.t. (328.3 ± 30.4 s) was similar to that observed in non-diabetic mice treated with fenvalerate at a dose of 3 μ g, i.t. (277.6 ± 27.4 s). Therefore, in subsequent experiments, mice were observed for 30 min after the injection of fenvalerate (non-diabetic mice: 3 μ g, diabetic mice: 0.1 μ g).

Effects of mexiletine on fenvalerate-induced nociceptive response

Figure 1A shows the effect of mexiletine on fenvalerate-induced behavior in non-diabetic and diabetic mice. Pretreatment with mexiletine (3, 10, and 30 mg/kg, i.p.) dose-dependently inhibited fenvalerate-induced nociceptive behavior in both non-diabetic mice [$F_{3,36} = 10.4$, $P < 0.01$] and diabetic mice ($F_{3,35} = 15.5$, $P < 0.01$). The % inhibition of the fenvalerate-induced

nociceptive response produced by i.p. mexiletine, at doses of 3, 10, and 30 mg/kg, in diabetic mice was significantly greater than those in non-diabetic mice (3 mg/kg: $F_{1,15} = 13.5$, $P < 0.01$; 10 mg/kg: $F_{1,18} = 17.3$, $P < 0.01$; 30 mg/kg: $F_{1,21} = 14.3$, $P < 0.01$). Indeed, the dose-response curve for % inhibition of the fenvalerate-induced nociceptive response produced by i.p. mexiletine in diabetic mice was shifted to the left of that in non-diabetic mice (Fig. 1B). The potency ratio (95% confidence limit) of the antinociception induced by mexiletine in diabetic mice vs non-diabetic mice was 7.00 (1.80 – 1078.52).

Effect of mexiletine on fenvalerate-induced thermal allodynia and hyperalgesia

As shown in Fig. 2, in vehicle-treated mice, the heat intensity at 35 V did not cause a tail-flick response within the 30-s limit. However, when the voltage of the bulb was increased to 50 V, the mean tail-flick latency was significantly less than 30 s. When non-diabetic mice were pretreated with fenvalerate, at a dose of 1 μ g, the mean tail-flick latencies at 35 ($F_{3,37} = 173.8$, $P < 0.01$) and 50 V ($F_{3,35} = 71.2$, $P < 0.01$) were significantly reduced.

As shown in Fig. 2, i.p. pretreatment with mexiletine (10 and 30 mg/kg) significantly reversed the fenvalerate-induced reduction of tail-flick latencies at 35 ($F_{2,28} = 129.7$, $P < 0.01$) and 50 V ($F_{2,28} = 89.2$, $P < 0.01$).

Discussion

In the present study, we found that i.t.-administered fenvalerate produced a characteristic behavioral response that mainly consisted of hind limb scratching directed toward caudal parts of the body and biting or licking of the hind legs. The intensity of fenvalerate-induced nociceptive responses was significantly greater in diabetic mice than in non-diabetic mice. Indeed, the duration of the nociceptive behavioral response induced by fenvalerate, at a dose of 0.1 μ g, i.t., was similar to that observed in non-diabetic mice treated with fenvalerate at a dose of 3 μ g, i.t. The present study also showed that fenvalerate-induced behavior was dose-dependently reduced by mexiletine (3, 10, and 30 mg/kg, i.p.) in both non-diabetic and diabetic mice. The antinociceptive potency of mexiletine toward fenvalerate-induced nociception in diabetic mice was significantly greater than that in non-diabetic mice. The potency ratio (95% confidence limit) of the antinociception induced by mexiletine in diabetic mice vs non-diabetic mice was 7.00. In the present study, we also examined the effect of mexiletine on fenvalerate-induced thermal allodynia and hyperalgesia, same as what was observed in diabetic mice, to confirm whether

Fig. 1. Effects of mexiletine on the fenvalerate-induced nociceptive response in non-diabetic and diabetic mice. A: Effects of mexiletine on the total duration of the fenvalerate-induced nociceptive response. Data are expressed as the total duration of the response during the 30-min period after injection. Mexiletine (3, 10, and 30 mg/kg) was injected i.p. 30 min before the injection of fenvalerate (non-diabetic mice: 3 μ g, i.t., diabetic mice: 0.1 μ g, i.t.). Each column represents the mean with S.E.M. for at least 10 mice in each group. * P <0.05 vs respective vehicle-treated group. B: Dose-response curves of the effect of mexiletine on the nociceptive response induced by the i.t. administration of fenvalerate in non-diabetic (open circle) and diabetic mice (closed circle). Data were expressed as the % inhibition of the nociceptive response [(saline-treated value – drug-treated value)/ saline-treated value $\times$ 100]. Each circle represents the mean with S.E.M. for at least 10 mice in each group. * P <0.05 vs respective non-diabetic group.

the inhibitory effect of TTX-R contributed to action of mexiletine. The present results showed that pretreatment with mexiletine (10 and 30 mg/kg) dose-dependently reversed the fenvalerate-induced thermal hyperalgesia and allodynia in the tail-flick test in naive mice. Our previous studies strongly suggested that the activation of TTX-R sodium channels is necessary to produce a fenvalerate-induced nociceptive response and thermal allodynia/hyperalgesia (27, 28). Furthermore, we also suggested that the sensitization of sodium channels, probably TTX-R sodium channels, by the long-term activation of protein kinase C may play an important role in the enhancement of the duration of fenvalerate-induced nociceptive behavior in diabetic mice (25). We

Fig. 2. Effect of mexiletine on the fenvalerate-induced thermal allodynia and hyperalgesia in naive (non-diabetic) mice. Mexiletine (10 and 30 mg/kg) and its vehicle (saline) were injected i.p. 30 min before the injection of fenvalerate. Fenvalerate (1 μ g) was injected i.t. 60 min before testing. Each point represents the mean with S.E.M. for at least 10 mice in each group. * P <0.05 vs respective vehicle-treated group (open column). # P <0.05 vs fenvalerate alone-treated group (hatched column).

previously reported that streptozotocin-induced diabetic mice showed thermal allodynia and hyperalgesia in the tail-flick test (24, 34, 35). Indeed, in non-diabetic mice, the high heat intensity at bulb voltages of 65 and 90 V evoked a rapid tail-flick response, whereas that at 50 V evoked an intermediate tail-flick latency, and that at 25 and 35 V evoked no tail-flick response (24, 34, 35). In diabetic mice, the tail-flick latency after heating the tail at 50 V was significantly shorter than that in non-diabetic mice, indicating that diabetic mice exhibit thermal hyperalgesia. In addition, a lower voltage (35 V), which did not evoke a tail-flick response in non-diabetic mice, evoked a tail-flick response in diabetic mice, indicating that diabetic mice exhibit thermal allodynia (24, 34, 35). Furthermore, we also suggested that the thermal allodynia and hyperalgesia seen in diabetic mice may be due to enhancement of the release of substance P from capsaicin-sensitive primary afferent fibers in the spinal cord (24, 34, 35). In this regard, we reported that the tail-flick latency after heating at 35 V in diabetic mice was dose-dependently increased by i.p. pretreatment with mexiletine (35). Furthermore, when mice were pretreated with mexiletine (30 mg/kg, i.p.), there was no significant difference between the tail-flick latencies at 50, 65, and 90 V in both diabetic and non-diabetic mice (35). These results indicate that mexiletine has anti-allodynic and anti-hyperalgesic effects in diabetic mice. Interestingly, the anti-hyperalgesic effect of mexiletine in diabetic mice was markedly reduced when diabetic mice were pretreated with capsaicin, which prolonged the latency of the tail-flick response at a lower heating rate (50 V) to the level of that observed in non-diabetic mice (35). However, the same procedure had no significant effect on the antinociceptive effect of mexiletine in non-diabetic mice (35). Furthermore, the fenvalerate-induced nociceptive response was found to be inhibited by GR82334, a non-peptidic NK-1-receptor antagonist (28). Thus, we suggested that the anti-allodynic and anti-hyperalgesic effects of mexiletine may be due to the inhibition of the nociceptive transmission of capsaicin-sensitive primary afferent fibers (35). Based on the results of our previous and present studies, it seems likely that the antinociceptive effects of mexiletine may be, in part, due to the reduction of the nociceptive transmission of capsaicin-sensitive primary afferent fibers through the inhibition of TTX-R sodium channels.

In conclusion, our findings indicate that the antinociceptive effect of mexiletine on painful diabetic neuropathy may be, at least in part, mediated by the inhibition of TTX-R sodium channels-mediated nociceptive transmission in the spinal cord.

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